

**SEASONAL VARIATION IN THE LEAF ESSENTIAL OIL OF
TAXODIUM DISTICHUM (CUPRESSACEAE)**

Robert P. Adams

Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA
email Robert_Adams@baylor.edu

ABSTRACT

The leaf essential oil of *Taxodium distichum* is dominated by α -pinene (63-69%) with moderate amounts of limonene, β -phellandrene, myrcene and β -pinene. Oil yield increased from April (3.45 mg/g DM) to May (6.64) then rapidly declined to a somewhat steady state in the summer and fall. The major component, α -pinene, exhibited no significant variation on a percent total oil basis, but did vary on a mg/g DM basis. Caryophyllene oxide and germacrene D reached a maximum in June and July, respectively, then declined in the fall (Sep, Oct). Several components declined as percent total oil from early leaf set (Apr) to leaf yellowing (Oct): β -pinene, myrcene, limonene, β -phellandrene, terpinolene and (E)-caryophyllene. Three terpene acetates and a sesquiterpene aldehyde increased to their maximum levels in Sep-Oct. Seasonal variation in the constituents was not statistically different between % total oil and mg/g DM basis. *Phytologia* 94(1):91-102 (April 2, 2012).

KEY WORDS: *Taxodium distichum*, leaf essential oils, seasonal variation.

The composition of leaf essential oils can be influenced by season, rainfall, sunlight and other growth factors (von Koenen, 2001). In peppermint, Burbott and Loomis (1967) found that monoterpenes were stored for energy sources and utilized as needed. Adams (1970) found the leaf oil of *Juniperus pinchotii* varied from summer to winter with more variation in the summer than winter. Powell and Adams (1973) found that significant seasonal differences in the oil components

of *Juniperus scopulorum* were correlated with growth, temperature, and yield of oil. Seasonal variation on a g/g dw basis was more variable than on a percent basis (Powell and Adams, 1973). Adams and Hagerman (1976) examined the volatile leaf oil of *J. scopulorum* in young and mature leaves from the same greenhouse grown trees. They found 19 of the 36 compounds examined showed significant differences between young and mature leaves. Shanjani et al. (2010) examined seasonal changes in the volatile oils from both leaves and seed cones of *J. excelsa* (later shown to be *J. polycarpos*, Adams and Shanjani, 2011) in Iran. They found considerable seasonal variation in the seed cone oils with much less variation in the leaf oils. In general, in conifers, it appears that sampling leaves for volatile leaf oils for chemosystematic studies is less variable if done in during the fall and winter seasons.

However, not all conifers have leaves that persist throughout the year. *Taxodium* is a conifer genus in which the leaves are deciduous (except in mild, sub-tropical and tropical sites). *Taxodium distichum* (L.) Rich., bald cypress, is a common tree that grows from Texas to Florida along rivers. It is very commonly cultivated. Several trees are cultivated on Lake Tanglewood, TX in the Texas Panhandle. The leaves on these trees appear in April and generally turn yellow in October. Thus, these cultivated trees present an excellent opportunity to study the seasonal accumulation and changes of terpenes in their leaf oils. The purpose of this study was to determine the changes in the leaf essential oil of *T. distichum* throughout the growing season in the Texas Panhandle.

There has been a surprisingly little amount of research on the leaf essential oils of *Taxodium*. Odell (1912) reported on the volatile oil from the seed cones of *T. distichum* and Flamini et al. (2000) examined the essential oils from seed cones, leaves and branches from cultivated trees of *T. distichum* in North Tuscany, Italy. El Taunawy et al. (1999) reported that the essential oil from seed cones of *T. distichum* grown in Giza, Egypt. Ogunwande et al. (2007) reported the oils of *T. distichum* seed cone and leaves from a tree cultivated in Ibaden, Nigeria. The composition of *T. distichum* and *T. mucronatum* trees native to the western hemisphere has recently been reported (Adams et al., 2012).

MATERIALS AND METHODS

Plant material - *T. distichum*, Adams 12730, 12441, 318 N. Shore Dr., Lake Tanglewood, Amarillo, Randall Co., TX. A voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (200 g.) and air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate and Principal Component analyses were performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The leaf essential oil of *T. distichum* is dominated by α -pinene (63-69%, Table 1) with moderate amounts of limonene, β -phellandrene, myrcene and β -pinene. Oil yield increased from April (3.45 mg/g DM) to May (6.64) then rapidly declined to a somewhat steady state in the summer and fall (Table 1, Fig. 1). Interestingly, the major component, α -pinene, exhibited no significant variation on a percent total oil basis

($F= 0.9$, ns, Table 2), but did vary significantly on a mg/g DM basis (Table 3). Caryophyllene oxide showed a lag correlation with oil yield reaching a maximum (3.8%) in June, then declining (Table 1, Fig. 1). Germacrene D has a similar pattern reaching a maximum in August, then declining (Table 1, Fig. 1). Several components declined as percent total oil from early leaf set (Apr) to leaf yellowing (Oct): β -pinene, myrcene, limonene, β -phellandrene, terpinolene and (E)-caryophyllene (Table 1). These patterns are shown in Figure 2, with (E)-caryophyllene and terpinolene declining during summer growth. Myrcene was stable in April-May-June, then declined in July, and was again stable in Aug-Sep-Oct. A third pattern is shown in Fig. 3, with trans-pinocarvyl acetate rapidly increasing in the fall (Sep-Oct) along with the cis isomer (cis-pinocarvyl acetate) to a lesser degree (Fig. 3). Germacra-4(15),5,10(14)-trien-1-al produced a slightly different pattern (Fig. 3), increasing in the late summer, then with a significant decline in October.

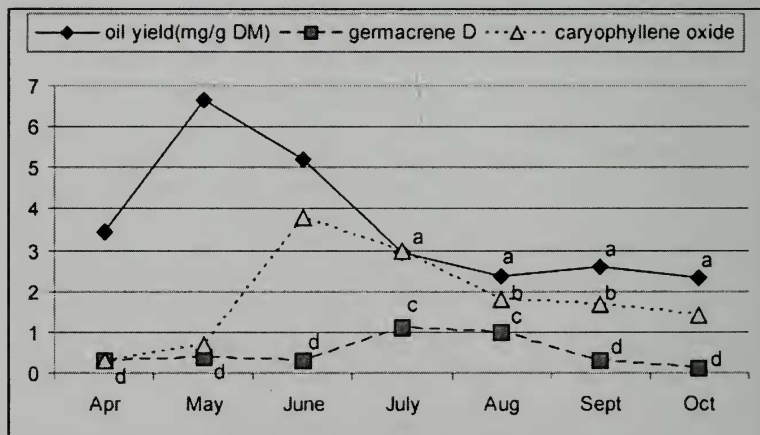


Figure 1. Seasonal variation in oil yield (mg/g DM), germacrene D and caryophyllene oxide (% total oil basis). Data points that share the same letter are not significantly different by the SNK test ($P=0.05$).

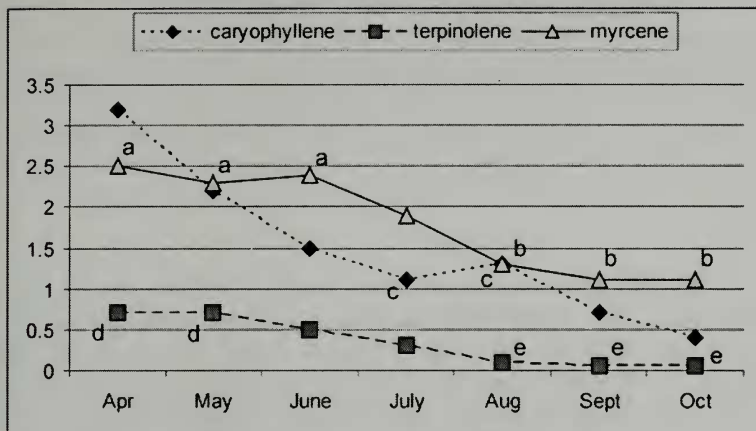


Figure 2. Seasonal variation in (E)-caryophyllene, terpinolene and myrcene (% total oil basis). Data points that share the same letter are not significantly different by the SNK test (P=0.05).

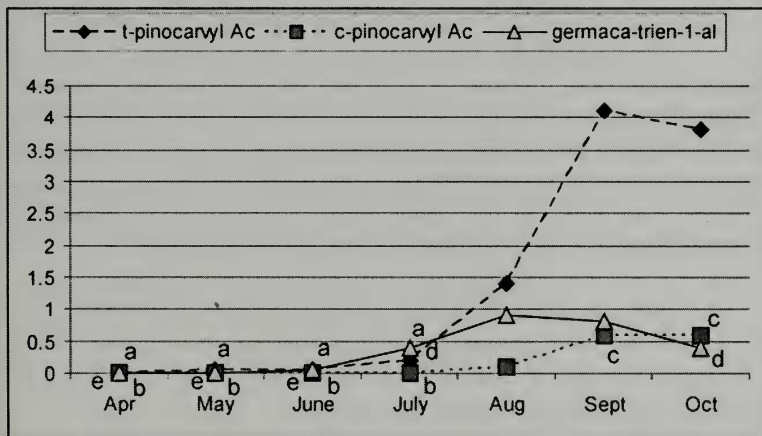


Figure 3. Seasonal variation in trans- and cis- pinocarvyl acetate and germaca-4(15),5,10(14)-trien-1-al (% total oil basis). Data points that share the same letter are not significantly different by the SNK test (P=0.05).

ANOVA, using oil yield (mg/g DM) and 15 major components (as % total oil), revealed that all exhibited highly significant seasonal differences except α -pinene (Table 2). The percent of α -pinene remained from 63.5 to 69.2% throughout the growing season, even in the yellowing October leaves. The average F value was 117.7. The pattern of trans- and cis- pinocarvyl acetate, trans-carvyl acetate and germacra-4(15),5,10(14)-trien-1-al are quite apparent in Table 2.

The data were recomputed on a mg/g DM basis and ANOVA performed. This resulted in an average F value of 116.6 that was not significantly different from the average F based on % total oil data (Table 2). Overall, the trends in the data expressed as mg/g DM are similar (Table 3) to the results found using % total oil (Table 2).

In order to examine the correlation patterns among terpenes and oil yield, Principal Component Analysis (PCA) was performed on the % total oil data. As expected, the terpene acetates (trans- and cis-pinocarvyl acetate, trans-carvyl acetate) were highly correlated as seen in Figure 4. Oil yield was correlated with β -pinene and limonene (Fig. 4). The components that decreased as oil yield decreased in the season (β -pinene, limonene, β -phellandrene, terpinolene, myrcene, caryophyllene) cluster (show a positive correlation) with oil yield (Fig. 4). In contrast, components that increased as oil yields declined are negatively correlated with oil yield and cluster at the far end of axis 1 (Fig. 4). Interestingly, PCA using mg/g DM data gave essentially the same ordination as using percent data (results not shown).

Notice that 85% of the variance among components was removed in the first three axes in PCA. This seems to imply that there may be only a few dominant biosynthetic pathways that control the terpene pattern in this particular *Taxodium*.

To examine the overall similarities among samples, PCO was performed on the similarity matrix. Ordination reveals that the samples cluster by date of collection, except for the Sep-Oct samples that seem to form a continuum (Fig. 5). PCO divides the collections into three

groups: spring - early summer (Apr-Jun), summer (Jul-Aug), and fall (Sep - Oct). The fall group appears to be the most uniform (Fig. 5). Fall appears to be the best time to sample for chemosystematic studies, but an early freeze would likely prove to be a serious problem.

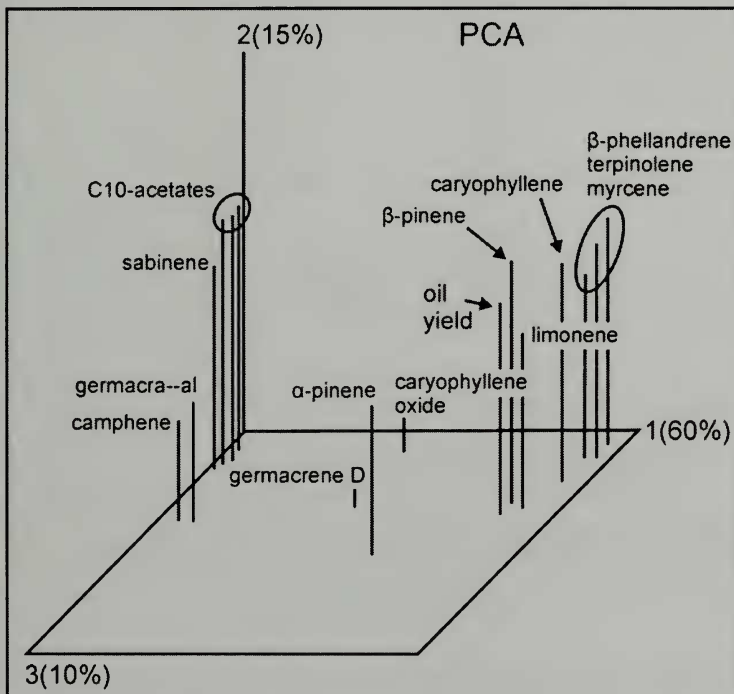


Figure 4. PCA of 21 samples using 16 characters.

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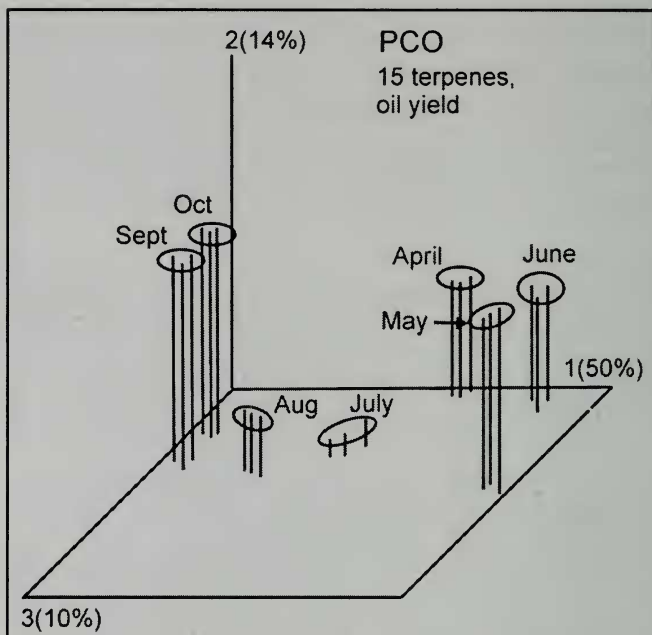


Figure 5. PCO showing samples cluster by dates of collection.

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Table 1. Composition of volatile leaf oils by collection dates for *T. distichum*. KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t).

KI	compound	Apr	May	June	July	Aug	Sept	Oct
	oil yield (mg/ g DM)	3.45	6.64	5.20	2.94	2.37	2.60	2.32
921	tricyclene	0.2	0.3	0.2	0.4	0.3	0.2	0.3
924	α -thujene	t	t	t	t	t	t	t
932	α -pinene	66.4	69.2	63.5	67.1	65.7	64.2	69.2
946	camphene	0.6	0.8	0.9	1.1	1.1	1.2	1.2
969	sabinene	0.5	0.2	0.1	0.2	0.7	0.8	0.9
974	β -pinene	1.4	0.9	0.9	0.9	0.9	0.8	0.8
988	myrcene	2.5	2.3	2.4	1.9	1.3	1.1	1.1
1014	α -terpinene	0.1	t	0.5	t	t	t	t
1020	p-cymene	t	t	t	t	0.1	0.2	0.2
1024	limonene	11.0	10.0	10.0	9.5	8.9	8.2	8.4
1025	β -phellandrene	10.0	9.6	9.4	6.8	6.0	7.2	5.6
1054	γ -terpinene	0.2	0.2	t	t	0.1	0.1	t
1086	terpinolene	0.7	0.7	0.5	0.3	0.1	t	t
1122	α -campholenal	-	-	-	-	-	t	t
1135	trans-pinocarveol	-	-	-	-	-	0.1	0.1
1137	trans-verbenol	-	-	-	-	t	t	t
1160	pinocamphone	-	-	-	-	t	0.1	t
1166	p-mentha-1,5-dien-8-ol	-	-	-	-	t	t	t
1174	terpinen-4-ol	0.2	0.1	t	t	t	t	t
1186	α -terpineol	0.4	0.4	0.3	0.2	0.1	t	t
1200	dodecane	t	-	t	t	0.1	0.1	0.1
1287	bornyl acetate	0.3	0.3	0.4	0.4	0.6	0.6	0.8
1298	trans-pinocarvyl acetate	-	t	t	0.2	1.4	4.1	3.8
1311	cis-pinocarvyl acetate	-	-	-	-	0.1	0.6	0.6
1324	myrtenyl acetate	-	t	-	t	0.2	0.4	0.3
1339	trans-carvyl acetate	-	t	-	t	0.2	0.5	0.4
1346	α -terpinyl acetate	-	t	-	t	t	t	t
1365	cis-carvyl acetate	-	-	-	-	t	t	t
1396	duvalene acetate	-	-	t	t	0.1	0.1	t
1417	(E)-caryophyllene	3.2	2.2	1.5	1.1	1.3	0.7	0.4
1431	β -copaene	-	t	t	t	t	0.1	t
1452	α -humulene	0.3	0.3	t	0.1	0.1	t	t
1478	γ -muurolene	-	-	-	t	t	t	t
1480	germacrene D	0.3	0.4	0.3	1.1	1.0	0.3	0.1
1513	γ -cadinene	-	t	t	t	t	t	t
1522	δ -cadinene	t	0.1	t	t	0.1	0.1	t
1582	caryophyllene oxide	0.3	0.7	3.8	3.0	1.8	1.7	1.4
1608	humulene epoxide II	t	t	0.1	0.4	0.4	0.3	0.2

